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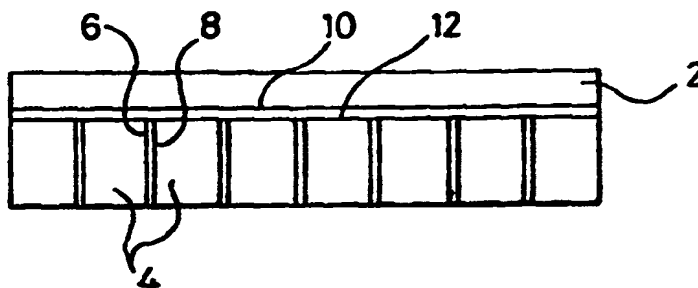
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : G01N 27/447</p>	<p>A1</p>	<p>(11) International Publication Number: WO 99/12025 (43) International Publication Date: 11 March 1999 (11.03.99)</p>
<p>(21) International Application Number: PCT/GB98/02587 (22) International Filing Date: 27 August 1998 (27.08.98) (30) Priority Data: 9718320.6 1 September 1997 (01.09.97) GB (71) Applicant (for all designated States except US): MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1N 4AL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): AHMADI, Alireza [IR/GB]; 27 Horsecraft Road, Edgware, Middlesex HA8 0PB (GB). BARON, Laurent [FR/FR]; 20, rue Edgar Quinet, F-94320 Thiais (FR). (74) Agent: KEITH W. NASH & CO.; 90-92 Regent Street, Cambridge CB2 1DP (GB).</p>		<p>(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.</p>

(54) Title: GEL LOADING

(57) Abstract

Disclosed is a gel loading aid comprising a porous solid support to which a plurality of samples may be reversibly immobilised, the support having a plurality of discrete sample sites, each site receiving a respective reversibly immobilised sample into the pores of the support, porous communication between adjacent samples sites being prevented by blockage of the pores therebetween, wherein said blockage is effected by crushing of the pores and/or filling of the pores with a blocking substance which prevents passage of the sample therethrough.



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Title: GEL LOADING

Field of the Invention

This invention relates to a gel loading aid comprising a porous solid support for receiving samples, a method of making said support, and a method of loading samples onto a gel

Background of the Invention

Presently, considerable research efforts world-wide are directed to the acquisition of nucleic acid sequence information. The Human Genome Mapping Project (HGMP) is one particularly well-known aspect of this research. The great scale of such sequencing projects has created a demand for improved DNA sequencing techniques which can process large numbers of samples per unit time.

Frequently one of the rate-limiting steps in the sequencing of samples is the process of physically introducing the samples onto the acrylamide gel, in which the samples are to be electrophoretically analysed. Sequencing gels are generally very thin (to improve resolution) and sample volumes quite small, so that the gel loading step is delicate and time consuming, and errors are not uncommon. Conventionally samples are loaded manually onto the gel via a micropipette, adjacent samples being kept separated by the presence of a thin, toothed comb made of plastics material, (commonly known as a "shark's teeth comb"), or by teeth pre-cast when the gel is poured.

A particular problem is the desire to run as many samples as possible on a single gel, and the conflicting need to have clear separation between adjacent samples to facilitate analysis of the results of the gel electrophoresis.

Various attempts have been made to produce automated methods and devices for gel loading, but none has so far proved generally acceptable: typically blocking of the narrow capillary tubing associated with such devices occurs with high frequency. Apparently one

such device is thought to comprise a membrane material onto which samples may be reversibly bound. The membrane is shaped like a conventionally-toothed comb, and placed on top of a gel. When an electrophoretic current is applied, the reversibly bound nucleic acid samples dissociate from the teeth of the membrane and are then resolved in the gel in the usual manner. Accordingly, the device relies on a toothed shape to maintain separation of the samples during transfer into the gel, which shape requires precision engineering to manufacture on a large scale.

Summary of the Invention

In a first aspect the invention provides a gel loading aid comprising a porous solid support to which a plurality of samples may be reversibly immobilised, the support having a plurality of discrete sample sites, each site receiving a respective reversibly immobilised sample into the pores of the support, porous communication between adjacent sample sites being prevented by blockage of the pores therebetween, wherein said blockage is effected by crushing of the pores and/or filling of the pores with a blocking substance which prevents passage of the sample therethrough.

In a second aspect the invention provides a method of forming a plurality of discrete sample sites on a porous solid support, the method comprising the step of creating a blocked-pore zone between each adjacent sample site, the blocked-pore zone being sufficient to prevent porous communication between respective samples applied to adjacent sample sites, wherein blocking of the pores in the blocked-pore zone is accomplished by crushing the pores and/or filling of the pores with a pore-blocking substance.

In a preferred embodiment the solid support comprises a porous membrane suitable for the reversible immobilisation of nucleic acid samples. In such embodiments, the solid support preferably comprises materials such as polyethersulfone. Suitable membranes are available, under the trade marks Supor™, GBI™ and BM™, from Gelman Sciences, Inc. Supor™ is particularly preferred and is a high performance membrane with a pore size of 0.1 μm and a thickness of 0.12mm. Other suitable membranes are available from Genetic Biosystems Inc. and from Boehringer Mannheim GmbH. For example, Boehringer

Mannheim produce a positively-charged "Nylon 66" microporous nylon membrane (with a 0.45 μm pore size) on a polyester support.

In some embodiments, crushing alone is sufficient to block the pores of the solid support. For large scale manufacture, machines suitable for crushing the pores of the solid support are commercially available. The solid support may be processed, for example, by crushing between rollers or, less preferably, by a stamping machine.

In other embodiments, no crushing is employed, and the pore blockage is instead accomplished solely by use of a blocking substance. Advantageously, the pores will be at least partly blocked by crushing, and at least partly blocked by a blocking substance. A particularly convenient method of achieving such "combined" blocking is by the use of pen or other rigid device suitable for applying a blocking substance. The pen can be applied to the surface of the solid support with sufficient pressure to crush the pores between adjacent sample sites. Simultaneously, the ink, paint, dye or other blocking substance with which the pen is supplied, flows into the pores of the membrane to assist in forming a blockage. The present inventors have been able to obtain satisfactory results using conventional ball point pens and conventional ink, but automated methods of applying a blocking substance are preferred. For example, the inventors have found that a dot matrix printer can very conveniently be used, which both crushes the pores of the membrane and applies ink.

The application of at least some pore-blocking substance is generally to be preferred, as it allows a user of the gel loading aid readily to identify the location of the blocked-pore zones, and thus the location of the sample sites, which might not be readily apparent if crushing alone is used to create the blocked-pore zones. For this reason it is advantageous that at least some of the pore blocking substance should be opaque and typically coloured so as to be easily distinguishable against the gel loading aid. Alternatively, if crushing alone is relied upon to form the blocked-pore zone, then a non-pore blocking substance may conveniently be applied to the gel loading aid in order to enable a user to identify the location of the sample sites.

Where a pore-blocking substance is employed, it will generally be advantageous to apply an excess amount. This will ensure that the pores in the intended blocked-pore zone are completely blocked. In addition, this may lead to the deposition of excess pore-blocking substance on the surface of the solid support. This has the advantage that the deposition on the surface of the membrane of a (typically) hydrophobic pore-blocking substance helps prevent the mixing of samples applied to the support e.g. where a pipette tip slips slightly during the application of the sample. It may also improve the ease with which a user can identify the location of the sample sites.

The nature of the blocking substance will be determined at least in part, by the nature of the solid support and the nature of the samples to be reversibly immobilised on the support. Typically the samples will be applied to the support in an aqueous solution, such that a hydrophobic blocking substance is desirable, the hydrophobic nature serving to prevent passage of the aqueous solution (comprising the sample) through the pores of the solid support. Examples of suitable blocking substances include inks, dyes and waxes. Preferably the ink, dye or other blocking substance is not water soluble, so that the use of aqueous solutions will not dissolve the blocking substance.

Where the solid support is very thin (e.g. less than 0.2mm) it is advantageous to provide a backing material to add mechanical strength and protect the support from damage (especially during the pore-blocking step in the manufacture of the gel loading aid). Conveniently the backing material is provided with an adhesive, compatible with the porous solid support, for sticking the backing material thereto. Conveniently the backing material comprises an adhesive tape such as Scotch™ tape, or a synthetic or natural paper (e.g. conveniently provided with an adhesive coating). It may also be desired to provide the gel loading aid with one or more handling areas, which comprise a region of relatively strong material which, not being used for reversible immobilisation of samples, may be of a non-porous nature. Conveniently a handling area is provided at each end of the gel loading aid.

In a preferred embodiment the gel loading aid comprises a physically continuous layer of porous material, within which are created blocked-pore zones, the zones serving to isolate

discrete sample sites and preventing mixing of different samples applied to respective sample sites. Samples, typically nucleic acid samples for analysis by acrylamide gel electrophoresis, may be reversibly immobilised on the solid support, each sample being reversibly immobilised at a respective sample site on the support. The gel-loading aid comprising such a solid support advantageously has dimensions which allow the aid to be positioned adjacent to the gel, in the gap between the (typically, glass) plates in which the gel is sandwiched. Desirably the gel-loading aid has a straight edge which may be positioned flush against the top edge of the gel. As with conventional gel-loading methods, the gel is immersed in an electrolytic buffer and a voltage applied across the gel. The reversibly immobilised samples on the solid support are then released from the support and drawn into the gel by electrophoresis. Mixing of adjacent samples after being released from the support, but prior to entry into the matrix of the gel, is prevented by the blockage of the pores between the sample sites. The aqueous nature of the electrophoresis buffer requires that in this embodiment, the pore-blocking substance, if used, is hydrophobic in nature to prevent the pores becoming unblocked by dissolution of the pore-blocking substance in the buffer.

Conveniently the blocked-pore zone forms a narrow strip between adjacent sample sites. This may be accomplished, for example, by pressing a pen nib hard onto the solid support and drawing a straight line. As described above, the use of a suitable pen and ink causes pore blockage by crushing and also simultaneous blocking of the pores by deposition of a pore blocking substance (the ink). For increased confidence that mixing of the samples cannot occur, a plurality of blocked-pore zones may be provided between adjacent sample sites. The present inventors have found that a pair of closely-spaced, parallel zones of ink, applied with a ball-point pen, confer complete separation for most purposes. If a still further degree of sample separation on the solid support is required, the number and/or width of the pore-blocked zones between adjacent sample sites can be increased. For example, adjacent sites could be separated by two parallel ink lines (forming two narrow blocked-pore zones, in which the pores are both crushed, and filled with ink), and the support between the ink lines could be filled with a second pore-blocking substance (e.g. a wax). This arrangement could be seen as providing one very wide, continuous blocked-pore zone or, more properly, as three separate blocked-pore zones. In addition, if

desired, the sample sites may be entirely defined by blocked-pore zones, rather than simply defining blocked-pore zones between adjacent sample sites. Thus, for example, each sample site could be substantially surrounded by blocked-pore zones, except for a neck or outlet by which the sample leaves the membrane and enters a gel.

A gel-loading aid in accordance with the present invention possesses several advantages over known methods or devices of loading gels. Firstly, compared to the conventional manual loading of samples directly onto gels, the present invention greatly facilitates the exercise: samples may be loaded onto the readily accessible solid support, rather than onto the comparatively inaccessible gel, with the result that loading can be accomplished much more quickly, and with fewer errors than previously. Further, the invention renders feasible a practical, successful and automated method of loading gels.

Further, whilst there have been previous attempts to use membranes as gel-loading aids, the membranes have been shaped like conventional gel combs, with teeth, to ensure separation of adjacent samples. In the present invention there is no need for teeth - accordingly a gel-loading aid is much simpler to manufacture, as providing teeth requires precision cutting of the membrane and makes the membrane prone to mechanical damage. In addition, a higher density of sample sites can be obtained, as adjacent sites can be positioned more closely than the teeth of a comb can conveniently be cut.

The present inventors have also investigated the storage of gel loading aids in accordance with the invention, with nucleic acid samples applied thereto. It has been found that allowing the samples to dry on the solid support tends to make the nucleic acid become irreversibly immobilised to the support, which is undesirable. Storage at ambient temperature under humid conditions (e.g. in a humid chamber) prevents drying of the samples, but allows diffusion within the sample site - this is generally undesirable as it tends to reduce resolution following gel electrophoresis. Accordingly, such storage methods are generally only suitable where the sample site is entirely defined by a pore-crushed margin, and where the site is of small area. Storage for a short period (about 30 minutes) at 4°C, without any other precautions, gives acceptable results. However, long term storage is best accomplished at sub-zero temperatures in freezers. The inventors

have found that samples may be reversibly immobilised on a solid support, and the support may be stored frozen for 24 hours prior to loading onto a gel, without any significant detrimental effect. It is however important to ensure that the samples do not dry when stored at -20°C, for example by wrapping them up in cling film.

The ability stably to store the support, bearing the samples, is highly desirable as it makes the whole process of preparing and loading samples more flexible, and more amenable to automation.

The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, in which:

Figure 1 shows a plan view of a portion of a gel loading aid in accordance with the invention; and

Figure 2 shows a plan view of a different gel loading aid in accordance with the invention.

Detailed Description of an Embodiment

Figure 1 shows a plan view of portion of a gel loading aid comprising a porous solid support 2 to which a plurality of nucleic acid samples may be reversibly immobilised. The Figure shows the support larger than actual size, for clarity. The embodiment illustrated is a gel-loading aid to facilitate the loading of nucleic acid samples onto an agarose DNA sequencing gel.

The gel-loading aid comprises the Supor™ (Gelman Sciences, Inc. USA) high performance polyethersulfone membrane. The membrane has a pore size of 0.1µm and is 0.12mm thick. The gel-loading aid further comprises a backing material, to which the membrane is bound. The backing material is a layer of adhesive Scotch™ tape, 0.06mm thick. The backing material increases the mechanical strength of the gel-loading aid, facilitates handling of the gel-loading aid, and protects the membrane from damage.

The gel-loading aid is 0.18mm (0.12mm + 0.06mm) thick and thus accommodated readily into the 0.2mm slot provided between the glass plates of a commercially available gel sandwich (Applied Biosystems, Inc. 377 glass plates, thin gel).

The solid support 2 is provided with a plurality of sample sites 4, each sample site 4 being adapted to receive a respective sample which is reversibly immobilised to the solid support. Adjacent sample sites 4 are separated by two narrow, parallel zones 6, 8 in which the pores of the solid support 2 have been blocked by crushing and by the deposition of a hydrophobic ink. The narrow zones are created by drawing a short line with a pen and applying pressure with the pen nib. Two further narrow, parallel zones 10, 12 run along the length of the membrane at right angles to the zones 6, 8 and define the top edge of the sample sites 4. The bottom edge of the sample sites 4 is defined by the edge of the membrane, which is positioned against the surface of the gel which is to be loaded with the samples reversibly immobilised on the solid support. Clearly, blocking of the pores along this bottom edge would prevent loading of the gel, such that delimiting the sample sites 4 on three sides is sufficient entirely to define the sample sites 4. Each sample site 4 is about 1.5mm wide and 5.5mm long.

The essentially rectangular solid support comprises 64 sample sites in an area 147mm long by 1.0cm wide. The solid support has a continuous, straight edge which facilitates positioning of the gel loading aid against the top of a gel to be loaded.

In use, a gel-loading aid is prepared by marking out the sample sites 4 on the membrane, as described above. Preferably the membrane is already provided with a backing material. Samples are then applied to the membrane. This can be done by automated means or manually. Typically samples are applied by absorption, in which a micropipette tip or capillary tube is immersed in the sample, and then positioned on a sample site 4, so transferring a small volume of the sample onto the membrane, to which the sample becomes reversibly immobilised, within the area of the sample site 4 defined by the zones of blocked pores (6, 8, 10, 12).

The process is repeated for each sample to be applied to the membrane. Alternatively,

especially if automated means for application of the samples is employed, a plurality of samples may be applied simultaneously, each sample being applied to a respective sample site 4.

Once all samples have been applied, the solid support may be used to load the samples onto a gel for analysis. If loading is not to take place immediately, the solid support can be stored for a short period (about 30 minutes) at room temperature or at 4°C, or for longer periods (24 hours or so) in a freezer at -20°C.

Loading of gels is very simply accomplished. A gel sandwiched between glass plates is held in a sequencing rig. The space between the two plates is then filled with a solution of linear acrylamide. (This avoids the formation or trapping of air bubbles, when inserting the comb, which could affect the electrophoresis and stops the possible diffusion of the samples out of the comb before the current is applied). The gel loading aid is then simply introduced between the glass plates onto the surface of the gel. The lower edge of the comb is linear and so sits flush against the top edge of the gel. The electrolytic buffer (TBE 1X: Tris Borate 89mM, Boric Acid 89mM, EDTA 2mM, pH 8.3) is then added and the current started, which creates an electric field forcing the DNA out of the comb and into the gel. Other suitable buffers having appropriate characteristics are well-known.

Once within the gel matrix, the molecular size of the samples, and the applied voltage, tends to keep the samples running down the gel in a straight line, preventing mixing. Mixing of the samples whilst still within the pores of the membrane (but released from the surface thereof) is prevented by the zones of pore blockage around each sample site 4.

The gel-loading aid described above is intended for use with the Applied Biosystems, Inc. 373 and 377 glass plate/thin gel arrangement. Those skilled in the art will appreciate that gel-loading aids of different dimensions can readily be prepared for use with gels of different sizes.

Figure 2 shows a plan view of another gel loading aid in accordance with the invention.

As with the embodiment shown in Figure 1, the gel loading aid comprises a porous solid support 2, provided with a plurality (sixty-four) of sample sites 4. Adjacent sample sites 4 are separated by two narrow, parallel zones 6, 8 in which the pores of the solid support 2 have been blocked by crushing and by the deposition of a hydrophobic ink. Each sample site 4 is 1.8mm wide and 10mm long. The zones 6, 8 are created by printing using a 24 pin dot matrix printer.

The solid support 2 is provided with an adhesive tape backing material, prior to dot matrix printing, in order to increase the mechanical strength of the support 2 and protect it from damage.

When the gel loading aid is applied to a gel, the electric field is such that the samples reversibly immobilised on the sample sites 4 are forced down the support, between the blocked-pore zones 6, 8, and into the gel. This unidirectional migration means that it is not essential to provide additional blocked-pore zones across the top of the sample sites 4 (as illustrated at 10 and 12 in Figure 1).

The gel loading aid is provided with a handling area 14 at one end, and another handling area 14' at the opposite end of the gel loading aid. The handling areas 14, 14' are made from relatively strong, synthetic plastics material, which may be non-porous.

CLAIMS

1. A gel loading aid comprising a porous solid support to which a plurality of samples may be reversibly immobilised, the support having a plurality of discrete sample sites, each site receiving a respective reversibly immobilised sample into the pores of the support, porous communication between adjacent sample sites being prevented by blockage of the pores therebetween, wherein said blockage is effected by crushing of the pores and/or filling of the pores with a blocking substance which prevents passage of the sample therethrough.
2. A gel loading aid according to claim 1, wherein the solid support comprises a porous membrane suitable for reversible immobilisation of nucleic acid samples.
3. A gel loading aid according to claim 1 or 2, wherein blockage of pores between adjacent sample sites is effected by crushing of the pores and by filling of the pores with a blocking substance.
4. A gel loading aid according to any one of claims 1, 2 or 3, wherein the pore blocking substance, if present, is opaque and defines visible blocked pore zones between adjacent sample sites on the solid support.
5. A gel loading aid according to any one of the preceding claims, wherein adjacent sample sites on the solid support are separated by a plurality of blocked pore zones.
6. A gel loading aid according to any one of the preceding claims, wherein the pore blocking substance is an ink, paint or dye.
7. A gel loading aid according to any one of the preceding claims, comprising a thin porous membrane attached to a backing material.
8. A gel loading aid according to claim 7, wherein the backing material is non-porous.

9. A method of forming a plurality of discrete sample sites on a porous solid support, the method comprising the step of creating a blocked-pore zone between each adjacent sample site, the blocked-pore zone being sufficient to prevent porous communication between respective samples applied to adjacent sample sites, wherein blocking of the pores in the blocked-pore zone is accomplished by crushing the pores and/or filling of the pores with a pore-blocking substance.
10. A method according to claim 9, performance of which method results in the formation of a gel loading aid in accordance with any one of claims 1-8.
11. A method of loading a plurality of samples onto a gel, the method comprising the steps of: loading a plurality of samples onto a gel loading aid in accordance with any one of claims 1-8; positioning the sample-bearing gel loading aid adjacent to the gel to be loaded; and applying an electrical potential difference, so as to cause the samples on the gel loading aid to migrate into the gel.
12. A method according to claim 11, wherein the sample-bearing gel loading aid is temporarily stored, prior to use, at a temperature below 0°C in a manner which prevents the samples becoming irreversibly immobilised on the solid support.

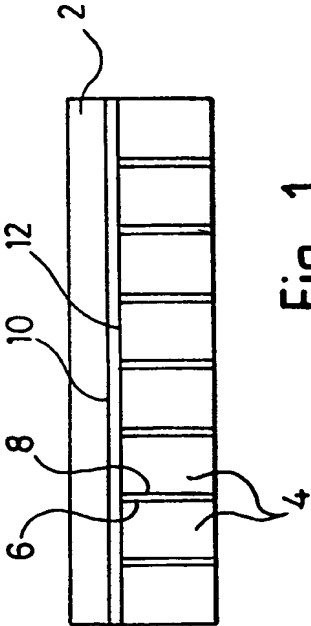


Fig. 1

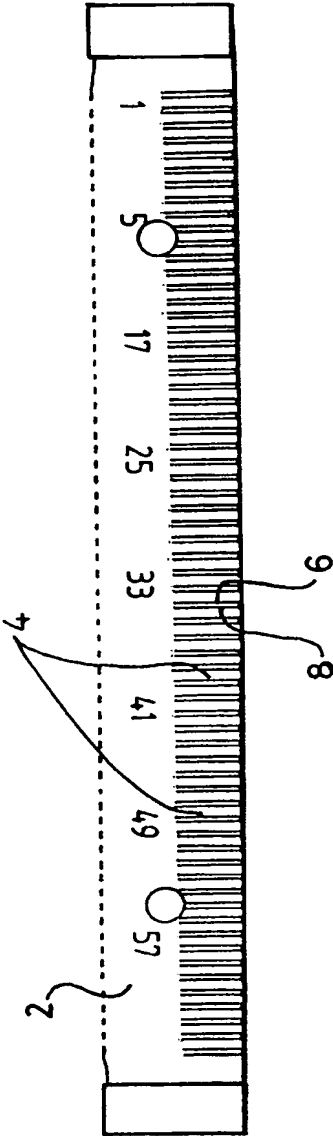


Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02587

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 27787 A (GENETIC BIOSYSTEMS INC) 12 September 1996 see abstract; figure 1B -----	1,9,11
A	US 5 464 515 A (BELLON FRANCK) 7 November 1995 see abstract; figure 1 -----	1
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

10 November 1998

Date of mailing of the international search report

17/11/1998

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INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/GB 98/02587

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